Method for Improvement of the Production of Adenovirus-based Vectors

Description

The invented method for improvement of the production of adenovirus-based vectors is characterized by gene transfer and overexpression of the cell cycle regulator $p21^{WAF1/CIP1}$ (p21), an inhibitor of cyclin-dependent kinases (CDK), in a production cell line.

As is well known, the methods in gene therapy are aimed at treating genetically based diseases by replacement of the defect genes by their intact wild type form. To do so, the wild type gene is transferred into the target tissue where this gene is normally expressed. For an effective therapy, the gene transfer has to reach a large proportion of the cells of the target tissue. With respect to efficacy, viral vectors to date are superior to nonviral vectors. The most commonly used viral vectors are adenovirus vectors (Ad vector).

The efficacy and quality of the amplification of adenovirus vectors in a production cell line critically depends on the time point when the cells are harvested. If the time point of cell harvest is too early then the yield of adenovirus-vector is to low, if the harvest is too late, the producing cells are already dead and, therefore, the adenovirus vector is lost. The latter is superimposed by the fact that amplification of the vector is accompanied by increased metabolic activity and nutrient consumption leading to acidification of the culture media which in turn is detrimental to the producing cells and, therefore, to the Ad vector.

Summary of the Invention

Therefore, the underlying concept of the invention was to develop a method that can stop the progress of this harmful mechanism allowing the Ad vector producing cells to survive.

The task is completed by the transfer of the gene or the cDNA of p21 into a given cell line used for the production of Ad vector, irrespective of the endogenous status of p21 in that cell line. Overexpression of p21 prevents apoptosis of the cells after infection with the Ad vector to be amplified and improves culture medium conditions.

Employing known gene transfer techniques the gene or the cDNA of the cell cycle regulator p21 is introduced as an expression cassette either with or without a regulatable promoter into a cell line for the production of Ad vector.

P21 is a known cell cycle regulator which prevents re-entry of senescent cells into cell cycle progression by blockage of cyclin-dependent kinases. This function includes different mechanisms like hypophosphorylation of the protein product of the Retinoblastoma Gene (Rb), binding to proliferating cell nuclear antigen (PCNA), binding to CDK-cyclin complexes like cyclin D-CDK4, cyclin E-CDK2, and cyclin A-CDK2. Whereas the interaction between p21 and PCNA prevents DNA replication, the interaction of p21 with cyclin dependent kinase complexes results in arrest of the cell cycle in the G_1-phase. The presence of p21 and of its cellular function is of vital importance for the survival of a cell. This importance is, for instance, illustrated by the fact, that there exist almost no mutations that are able to survive.

As known, eukaryotic cells replicate their genome only during a defined and limited period of time which is termed as phase of DNA synthesis (S-Phase) of the cell cycle. The cell cycle comprises four phases: G_1-phase, S-Phase, G_2-phase and Mitosis. The duration of each phase is rather constant. The G_1-phase lasts in fast proliferating cells between 2 and 20 hours, S-

Phase between 6 and 10 hours, G_2-phase between 2 and 4 hours and Mitosis between 3 and 4 hours.

For the transfer of p21 into already known production cell lines conventional gene transfer methods are used to transfer either the naked DNA or DNA packaged into vectors which can be of viral or non-viral nature.

According to the invention one application is to stably transfer the gene or the cDNA of p21 in conjunction with either a constitutive promoter or a regulatable promoter.

Another application is to transiently transfer the gene or the cDNA of p21 in conjunction with either a constitutive promoter or a regulatable promoter.

With respect to the invention a stable transfer is defined as integration of the expression cassette for p21 into the genome of the target cell whereas after a transient transfer the expression cassette for p21 remains epichromosomal.

The method according to the invention provides the advantage that expression of p21 in the production cell prolongs survival which allows harvesting of the Ad vector at the optimal time point.

The invention will be explained with the example of the amplification of different Ad vectors in a production cell line.

Ad vectors are amplified in specific production cell lines. Irrespective of the cell type used and the **method for cultivation** expression of the vector encoded transgene occurs already during replication of the Ad vector. In case of the expression of a toxic gene, e.g. a proapoptotic gene, the production cells die earlier; expression of an antiapoptotic gene extends the survival of the cells. While in the first instance Ad vector production is impaired, the latter instance has a benefical effect on Ad vector production. Consequently, production cells used to amplify an Ad vector which carries the apoptosis-promoting gene p53 (Ad.p53) will enter

apoptosis much earlier than when used to amplify an Ad vector carrying an antiapoptotic gene like p21 (Ad.p21) (Fig. 1). This is demonstrated by the number of dead cells as well as the metabolic parameter of the culture whereby the latter determine the quality of the Ad vector yield. Therefore, medium conditions of cells producing Ad.p21 are significantly better than those of cells infected with Ad.p53 (Fig. 2 to Fig. 4)

Figure Legends

Fig. 1 Morphology of cultures of the Ad vector production cell line 293 infected with different Ad vector. A: Mock infection, B: Ad.CD (Cytosine deaminase), C: Ad.p21, D: Ad.p53.

Fig. 2 Nutrient consumption as exemplified by the glucose concentration in cultures of the Ad vector production cell line 293 infected with different Ad vector (see Fig. 1).

Fig. 3 Cell damage as exemplified by lactate dehydrogenase (LDH) concentrations in the medium of cultures of the Ad vector production cell line 293 infected with different Ad vector (see Fig. 1).

Fig. 4 Lactate concentrations in the medium of cultures of the Ad vector production cell line 293 infected with different Ad vector (see Fig. 1).